

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1-14. (canceled).

15. (currently amended): A method for analyzing the C-terminal amino acid sequence of a peptide being maintained in a state that it is bound on a gel carrier, which method comprises steps of:

releasing the C-terminal amino acids successively from the peptide being bound on the gel carrier by chemical procedure to prepare a mixture containing said original peptide and a series of peptidyl reaction products produced therefrom,

analyzing the original peptide and said series of the peptidyl reaction products produced at the releasing step by means of mass spectrometry to measure the decreases in molecular weight associated with the successive release of the C-terminal amino acid, and

identifying a series of the C-terminal amino acids removed successively, based on a series of the measured decreases in molecular weight,

wherein the gel carrier is a polyacrylamide gel, and

the releasing step comprises:

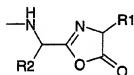
a sub-step (1) of conducting dehydration treatment for removing out the water solvent impregnated into the gel carrier,

a sub-step (2) of immersing the gel carrier, on which the peptide is still bound after said step of dehydration treatment, in a mixed solution of an alkanolic acid anhydride and a perfluoroalkanoic acid dissolved in a dipolar aprotic solvent to allow the alkanolic acid anhydride and the perfluoroalkanoic acid to act on the peptide being kept in the bound state; and

a sub-step (3) of removing the mixed solution from the gel carrier by dilution with use of a polar aprotic solvent;

wherein in the sub-step (2),

the successive release of the C-terminal amino acids results from the reaction process with use of the mixed solution in which formed is a 5-oxazolone-ring structure represented by the following general formula (III):



(III)

where R1 is a side chain of the C-terminal amino acid of the peptide and R2 is a side chain of the amino acid residue positioned just before the C-terminal amino acid, followed by the cleavage of the 5-oxazolone-ring.

16. (previously presented): A method claimed in Claim 15, wherein a concentration of the alkanolic acid anhydride contained in the mixed solution is selected in a range of 10 to 30% by volume.

17. (previously presented): A method claimed in Claim 15, wherein the sub-step (2) is carried out at a temperature selected in a range of 30 °C to 80 °C.

18. (previously presented): A method claimed in Claim 15, wherein a symmetric anhydride of an alkanolic acid having 2 to 4 carbon atoms is used as the alkanolic acid anhydride contained in said mixed solution.

19. (previously presented): A method claimed in Claim 15, wherein acetic anhydride is used as the alkanolic acid anhydride contained in the mixed solution.

20. (previously presented): A method claimed in Claim 15, wherein a perfluoroalkanoic acid of which a pKa is in the range of 0.3 to 2.5 is used as the perfluoroalkanoic acid contained in the mixed solution.

21. (previously presented): A method claimed in Claim 15, wherein a perfluoroalkanoic acid having 2 to 4 carbon atoms is used as the perfluoroalkanoic acid contained in the mixed solution.

22. (previously presented): A method claimed in Claim 15, wherein in the mixed solution, the content ratio of the alkanolic acid anhydride and the perfluoroalkanoic acid

is selected in the range of 1 to 20 volumes of the perfluoroalkanoic acid per 100 volumes of the alkanolic acid anhydride.

23. (previously presented): A method claimed in Claim 15, wherein, in the sub-step (2), the reaction system in which the alkanolic acid anhydride and the perfluoroalkanoic acid act on the peptide is kept in a dry atmosphere wherein not only water but also oxygen have been eliminated.

24. (previously presented): A method claimed in Claim 15, wherein the releasing step further comprises the following two substeps after the sub-step (3):

a sub-step (4) of hydrolysis treatment, in which the hydrolysis treatment for said mixture comprising the original peptide and the series of peptidyl reaction products is conducted by immersing the gel carrier in an aqueous solution dissolving a basic nitrogen-containing aromatic compound or a tertiary amine compound therein to allow a water molecule to act, in the presence of said basic nitrogen-containing organic compound, on the original peptide and the series of peptidyl reaction products being still bound on the gel carrier; and

a sub-step (5) of rehydration treatment, in which the rehydration treatment for the gel carrier is performed by removing said aqueous solution infiltrated into the gel carrier by dilution with use of a polar aprotic solvent.

25. (previously presented): A method claimed in Claim 15, wherein the releasing step further comprises the following two sub-steps for pretreatment before the sub-step (2):

a sub-step (6) of N-acylation protection, in which applying N-acylation protection to the N-terminal amino group of the peptide is conducted by immersing the gel carrier in a solution of an alkanolic acid anhydride dissolved in a dipolar aprotic solvent to allow the alkanolic acid anhydride to act on the peptide that is kept in the bound state; and

a sub-step (7) of termination of the N-acylation reaction, in which removal of said solution of the alkanolic acid anhydride is carried out by dilution with use of a polar aprotic solvent to conduct the termination of the N-acylation reaction.

26. (previously presented): A method claimed in Claim 15, wherein the peptide being maintained in a state that it is bound on the gel carrier has been in advance subjected to separation by gel electrophoresis.

27. (canceled).

28. (new): A method claimed in Claim 18, wherein a symmetric anhydride of a linear-chain alkanolic acid having 2 to 4 carbon atoms is used as said symmetric anhydride of an alkanolic acid having 2 to 4 carbon atoms.

29. (new): A method claimed in Claim 20, wherein a linear- chain perfluoroalkanoic acid having 2 to 4 carbon atoms is used as the perfluoroalkanoic acid having 2 to 4 carbon atoms.

30. (new): A method claimed in Claim 25, wherein the same alkanolic acid anhydride is employed for the alkanolic acid anhydride used in the sub-step (6) of N-acylation protection as well as for the alkanolic acid anhydride used in the sub-step (2).

31. (new): A method claimed in Claim 15, wherein a MALDI-TOF type mass spectrometry is selected as the mass spectrometry.